

We Claim:

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3/19/2000
- causing a desired trait
- SUB D1)
1. A method of making a localized mutation in a target gene in a plant cell comprising the steps of:
    - a. adhering to a particle a recombinagenic oligonucleobase, which contains a first homologous region which has a sequence identical to the sequence of at least 6 base pairs of a first fragment of the target gene and a second homologous region which has a sequence identical to the sequence of at least 6 base pairs of a second fragment of the target gene, and an intervening region which contains at least 1 nucleobase heterologous to the target gene, which intervening region connects the first homologous region and the second homologous region;
    - b. introducing the particle into a cell of a population of plant cells;
    - c. identifying a cell of the population cell having a mutation located between the first and second fragments of the target gene.

2. The method of claim 1, wherein the recombinagenic oligonucleobase is a MDON and each of the homologous regions contains an RNA segment of at least 6 RNA-type nucleotides.

3. The method of claim 2, wherein the intervening region is at least 3 nucleotides in length.

4. The method of claim 2, which further comprises the step of culturing the identified cell so that a plant is generated.

5. The method of claim 2, wherein the first RNA segment contains at least 8 contiguous 2'-Substituted Ribonucleotides.

6. The method of claim 5 wherein the second RNA segment contains at least 8 contiguous 2'-Substituted Ribonucleotides.

7. The method of claim 2, wherein the sequence of the mutated target gene is homologous with the sequence of the MDON.

8. The method of claim 2, wherein the adhering step is performed in a solution

comprising 1.1-1.4 M NaCl and 18-22  $\mu$ M spermidine and at least 14  $\mu$ g/ml

MDON.

9. The method of claim 2, wherein the target gene is a first ALS gene, a second ALS gene, a psbA gene, a threonine dehydratase gene, a dihydrodipicolinate synthase gene, or an S14/rp59 gene
- (10.) The method of claim 9, wherein the plant cell is a maize, wheat, rice or lettuce cell.
- 11.) The method of claim 9, wherein the plant cell is a potato, tomato, canola, soybean or cotton cell.
12. The method of claim 2, wherein the target gene selected from the group consisting of the genes encoding acid invertase, UDP-glucose pyrophosphorylase, polyphenol oxidase, O-methyl transferase, cinnamyl alcohol dehydrogenase, *etr-1* or a homolog thereof, ACC synthase and ACC oxidase.
13. The method of claim 12, where the plant cell is from a maize, wheat, rice or lettuce plant.
14. The method of claim 12, where the plant cell is from a potato, tomato, canola, soybean or cotton plant.
15. The method of claim 2, which further comprises making seeds from the plant or from progeny of the plant.
16. A method of making a localized mutation in a target gene in a plant cell having a cell wall comprising the steps of:
- a. perforating the cell walls of a population of plant cells;
  - b. introducing a recombinagenic oligonucleobase, which contains a first homologous region which has a sequence identical to the sequence of at least 6 base pairs of a first fragment of the target gene and a second homologous region which has a sequence identical to the sequence of at least 6 base pairs of a second fragment of the target gene, and an intervening region which contains at least 1 nucleobase heterologous to the target gene, which intervening region connects the first homologous region

*causing a desired trait*

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contg.

and the second homologous region;

identifying a cell of the population having a mutation located between the first and second fragments of the target gene.

17. The method of claim 16, wherein the recombinogenic oligonucleobase is a MDON and each of the homologous regions contains an RNA segment of at least 6 RNA-Type nucleotides.

18. The method of claim 17, which further comprises the step of culturing the identified cell so that a plant is generated.

19. The method of claim 17, wherein the sequence of the target gene between the first and the second fragments differs from the sequence of the intervening region of the MDON at a mismatched nucleotide and the mutation of the target gene is located adjacent to the mismatched nucleotide.

20. The method of claim 17, wherein the sequence of the target gene between the first and the second fragments differs from the sequence of the mutator segment of the MDON at a mismatched nucleotide and the mutation of the target gene is located at the mismatched nucleotide.

21. The method of claim 17, wherein the target gene is a first ALS gene, a second ALS gene, a psbA gene, a threonine dehydratase gene, a dihydrodipicolinate synthase gene, or an S14/rp59 gene

22. The method of claim 21, wherein the plant cell is a maize, wheat, rice or lettuce cell.

23. The method of claim 21, wherein the plant cell is a potato, tomato, canola, soybean or cotton cell.

24. The method of claim 17, wherein the target gene is selected from the group consisting of the genes encoding acid invertase, UDP-glucose pyrophosphorylase, polyphenol oxidase, O-methyl transferase, cinnamyl alcohol dehydrogenase, *etr-1* or a homolog thereof, ACC synthase and ACC oxidase.

25. The method of claim 24, where the target gene is a gene from a maize, wheat, rice or lettuce plant.

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(26.) The method of claim 24, where the target gene is a gene from a potato, tomato, canola, soybean or cotton plant.

(27.) The method of claim 17, which further comprises making seeds from the plant or from progeny of the plant.

*causing a desired trait*  
28. A method of making a localized mutation in a target gene of a plastid of a plant cell which comprises the steps of:

- a. Introducing a recombinagenic oligonucleobase, which contains a first homologous region which has a sequence identical to the sequence of at least 6 base pairs of a first fragment of the target gene and a second homologous region which has a sequence identical to the sequence of at least 6 base pairs of a second fragment of the target gene, and an intervening region which contains at least 1 nucleobase heterologous to the target gene, which intervening region connects the first homologous region and the second homologous region;
- b. Identifying a cell having a mutation in the region between the first and second fragments of the target gene.

29. The method of claim 28, wherein the recombinagenic oligonucleobase is a MDON and each of the homologous regions contains an RNA segment of at least 6 RNA-Type nucleotides.

30. The method of claim 29, which further comprises culturing the identified cell so that a plant is generated.

31. A method of making a localized, non-selectable mutation in a target gene in a plant cell comprising the steps of:

- a. introducing into the cells of a population of cells a mixture of a first recombinagenic oligonucleobase and a second recombinagenic oligonucleobase wherein:
  - i. the first recombinagenic oligonucleobase contains a first homologous region which has a sequence identical to the sequence of at least 6 base pairs of a first fragment of a first target gene and a second homologous

region which has a sequence identical to the sequence of at least 6 base pairs of a second fragment of the first target gene, and an intervening region which contains at least 1 nucleobase heterologous to the target gene, which intervening region connects the first homologous region and the second homologous region, and

- ii. the second recombinagenic oligonucleobase contains a first homologous region which has a sequence identical to the sequence of at least 6 base pairs of a first fragment of a second target gene and a second homologous region which has a sequence identical to the sequence of at least 6 base pairs of a second fragment of the second target gene, and an intervening region which contains at least 1 nucleobase heterologous to the target gene, which intervening region connects the first homologous region and the second homologous region;
  - b. selecting cells from the population having a selectable mutation located between the first and the second fragments of the first target gene from the population; and
  - c. identifying a selected cell having a non-selectable mutation located between the first fragment and the second fragment of the second target cell.
32. The method of claim 31, wherein the each recombinagenic oligonucleobase is a MDON and each of the homologous regions contains an RNA segment of at least 6 RNA-Type nucleotides.
33. The method of claim 32, wherein the first target gene is a first ALS gene, a second ALS gene, a psbA gene, a threonine dehydratase gene, a dihydrodipicolinate synthase gene, or an S14/rp59 gene.
34. The method of claim 33, wherein the plant cell is a maize, wheat, rice or lettuce cell.
35. The method of claim 33, wherein the plant cell is a potato, tomato, canola, soybean or cotton cell.

36. The method of claim 32, wherein the second target gene is selected from the group consisting of the genes encoding acid invertase, UDP-glucose pyrophosphorylase, polyphenol oxidase, O-methyl transferase, cinnamyl alcohol dehydrogenase, *etr-1* or a homolog thereof, ACC synthase and ACC oxidase.
37. The method of claim 36, wherein the plant cell is a maize, wheat, rice or lettuce cell.
38. The method of claim 36, wherein the plant cell is a potato, tomato, canola, soybean or cotton cell.
39. The method of claim 32, which further comprises culturing the identified cell such that a plant is generated.
40. The method of claim 39, which further comprises making seeds from the plant or from progeny of the plant.
41. The method of claim 31, wherein the second recombinogenic oligonucleobase is a heteroduplex recombinogenic oligonucleobase and each of the homologous regions of the second recombinogenic oligonucleobase contains an RNA segment of at least 6 RNA-Type nucleotides.
42. The method of claim 41, wherein the first target gene is a first ALS gene, a second ALS gene, a *psbA* gene, a threonine dehydratase gene, a dihydrodipicolinate synthase gene, or an S14/rp59 gene.
43. The method of claim 42, wherein the plant cell is a maize, wheat, rice or lettuce cell.
44. The method of claim 42, wherein the plant cell is a potato, tomato, canola, soybean or cotton cell.
45. The method of claim 41, wherein the second target gene is selected from the group consisting of the genes encoding acid invertase, UDP-glucose pyrophosphorylase, polyphenol oxidase, O-methyl transferase, cinnamyl alcohol dehydrogenase, *etr-1* or a homolog thereof, ACC synthase and ACC oxidase..
46. The method of claim 36, 45, wherein the second target gene is from a maize, wheat, rice or lettuce plant.

47. The method of claim 36, 45, wherein the second target gene is from a potato, tomato, canola, soybean or cotton plant.
48. The method of claim 41, which further comprises culturing the identified cell such that a plant is generated.
49. The method of claim 48, which further comprises making seeds from the plant or from progeny of the plant.

- ✓ 50. A method of making a localized mutation in a target gene in a plant cell comprising the steps of:
- digesting a plant part with cellulase such that plant cell protoplasts are formed;
  - suspending the protoplasts in a solution comprising a recombinagenic oligonucleobase which contains a first homologous region which has a sequence identical to the sequence of at least 6 base pairs of a first fragment of the target gene and a second homologous region which has a sequence identical to the sequence of at least 6 base pairs of a second fragment of the target gene, and an intervening region which contains at least 1 nucleobase heterologous to the target gene, which intervening region connects the first homologous region and the second homologous region;
  - electroporating the suspension such that the recombinagenic oligonucleobase enters a protoplast of the suspension;
  - culturing the protoplast; and
  - identifying a progeny of the protoplast having a mutation located between the first and second fragments of the target gene.
51. The method of claim 50, which further comprises the step of culturing the identified progeny such that a plant is generated.
52. The method of claim 50, wherein the recombinagenic oligonucleobase is a MDON and each of the homologous regions contains an RNA segment of at least 6 RNA-Type nucleotides.

53. The method of claim 50, wherein the recombinagenic oligonucleobase is an heteroduplex recombinagenic oligonucleobase.
54. A plant or seed having a point mutation in a gene is in its wild type genetic position, which gene is selected from the group consisting of the genes encoding acid invertase, UDP-glucose pyrophosphorylase, polyphenol oxidase, O-methyl transferase, cinnamyl alcohol dehydrogenase, ACC synthase and ACC oxidase or *etr-1* or a homolog of *etr-1*, and the sequence of the genomic DNA within 23 KB of the mutation is the sequence of the wild type DNA, and the point mutation forms a stop codon or is a frameshift mutation.
55. The plant or seed of claim 54, in which the point mutation forms a stop codon.
56. The plant or seed of claim 55, in which the sequence of the genomic DNA within 40 KB of the selectable mutation is the sequence of the wild type DNA.
57. The plant or seed of claim 55, in which the sequence of the genomic DNA within 100 KB of the selectable mutation is the sequence of the wild type DNA.
58. The plant or seed of claim 55, in which the point mutation is a single base pair mutation.
59. The plant or seed of claim 55, which is a maize, wheat, rice or lettuce plant or seed.
60. The plant or seed of claim 55, which is a potato, tomato, canola, soybean or cotton plant or seed.
61. The plant or seed of claim 55, further having a selectable point mutation in a second gene and the sequence of the genomic DNA within 23 KB of the selectable point mutation is the sequence of the wild type DNA.
62. The plant or seed of claim 61, in which the sequence of the genomic DNA within 40 KB of the selectable mutation is the sequence of the wild type DNA.
63. The plant or seed of claim 61, in which the sequence of the genomic DNA within 100 KB of the selectable mutation is the sequence of the wild type DNA.
64. The plant or seed of claim 54, in which the point mutation is a frameshift



mutation.

65. The plant or seed of claim 64, in which the sequence of the genomic DNA within 40 KB of the selectable mutation is the sequence of the wild type DNA.
66. The plant or seed of claim 64, in which the sequence of the genomic DNA within 100 KB of the selectable mutation is the sequence of the wild type DNA.
67. The plant or seed of claim 64, in which the point mutation is a single base pair mutation.
68. The plant or seed of claim 64, which is a maize, wheat, rice or lettuce plant or seed.
69. The plant or seed of claim 64, which is a potato, tomato, canola, soybean or cotton plant or seed.
70. The plant or seed of claim 64, further having a selectable point mutation in a second gene and the sequence of the genomic DNA within 23 KB of the selectable point mutation is the sequence of the wild type DNA.
71. The plant or seed of claim 70, in which the sequence of the genomic DNA within 40 KB of the selectable mutation is the sequence of the wild type DNA.
72. The plant or seed of claim 70, in which the sequence of the genomic DNA within 100 KB of the selectable mutation is the sequence of the wild type DNA.